

WHAT WE CLAIM IS:

1. A hybridization assay probe comprising an oligonucleotide which hybridizes to a target sequence present in nucleic acid derived from a *Cryptosporidium* organism in a test sample under stringent conditions to form a probe:target hybrid stable for detection, said oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in the target sequence, wherein the target sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, and wherein said probe does not hybridize to nucleic acid derived from a non-*Cryptosporidium* organism in the test sample to form a probe:non-target hybrid stable for detection under the stringent conditions.

2. The probe of claim 1, wherein said oligonucleotide has an at least 10 contiguous base region which is at least 90% complementary to an at least 10 contiguous base region present in the target sequence.

3. The probe of claim 1, wherein said oligonucleotide has an at least 10 contiguous base region which is 100% complementary to an at least 10 contiguous base region present in the target sequence.

4. The probe of claim 1, wherein said probe is up to 100 bases in length.

5. The probe of claim 1, wherein said probe is from 12 to 50 bases in length.

6. The probe of claim 1, wherein said probe is from 18 to 35 bases in length.

7. The probe of claim 1, wherein said probe contains base sequences which hybridize to each other when not hybridized to the target sequence under the stringent conditions.

5 8. The probe of claim 1, wherein said probe comprises one or more base sequences which do not stably hybridize to nucleic acid derived from *Cryptosporidium* organisms, or to nucleic acid derived from a non-target organism present in the test sample, under the stringent conditions.

10 9. The probe of claim 8, wherein said probe comprises two of said one or more base sequences, wherein said two base sequences hybridize to each other when said probe is not hybridized to said target sequence under the stringent conditions.

15 10. The probe of claim 1 further comprising a detectable label.

11. The probe of claim 1 further comprising a group of interacting labels.

12. The probe of claim 11, wherein said interacting labels include a luminescent label and a quencher label.

20 13. The probe of claim 1, wherein said oligonucleotide includes at least one ribonucleotide modified to include a 2'-O-methyl substitution to the ribofuranosyl moiety.

25 14. The probe of claim 1, wherein a pseudo peptide backbone joins at least a portion of the bases of said oligonucleotide.

30 15. The probe of claim 1, wherein the stringent conditions comprise 50 mM succinic acid, 1% (w/v) LLS, 7.5 mM aldrithiol-2, 0.6 M LiCl, 115 mM LiOH, 10 mM EDTA, 10 mM EGTA, 1.5% (v/v) ethyl alcohol (absolute), pH to 4.7, and a test sample temperature of about 60°C.

16. A hybridization assay probe comprising an oligonucleotide which hybridizes to a target sequence present in nucleic acid derived from a *Cryptosporidium* organism in a test sample under stringent conditions to form a probe:target hybrid stable for detection, wherein the base sequence of said oligonucleotide is at least 80% complementary to the base sequence of the target sequence, wherein the target sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, and wherein said probe does not hybridize to nucleic acid derived from a non-*Cryptosporidium* organism in the test sample to form a probe:non-target hybrid stable for detection under the stringent conditions.

10
15
16. An oligonucleotide probe which hybridizes to a target sequence present in nucleic acid derived from a *Cryptosporidium* organism in a test sample under stringent conditions to form a probe:target hybrid stable for detection, wherein the base sequence of said probe is at least 80% complementary to the base sequence of the target sequence, wherein the target sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, and wherein said probe does not hybridize to nucleic acid derived from a non-*Cryptosporidium* organism in the test sample to form a probe:non-target hybrid stable for detection under the stringent conditions.

20
25
18. An oligonucleotide probe which hybridizes to a target sequence present in nucleic acid derived from a *Cryptosporidium* organism in a test sample under stringent conditions to form a probe:target hybrid stable for detection, wherein the base sequence of said probe is fully complementary to the base sequence of the target sequence, wherein the target sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, and wherein said probe does not hybridize to nucleic acid derived from a non-*Cryptosporidium* organism in the test sample to form a probe:non-target hybrid stable for detection under the stringent conditions.

30
19. A probe mix comprising the probe of claim 1 and one or more helper

oligonucleotides having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in a target sequence, wherein the target sequence is selected from the group consisting of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27 and SEQ ID NO:28.

20. The probe mix of claim 19, wherein the target sequence of one of said helper oligonucleotides is selected from the group consisting of SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25 and SEQ ID NO:27.

10
21. The probe mix of claim 19, wherein the target sequence of one of said helper oligonucleotides is selected from the group consisting of SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26 and SEQ ID NO:28.

15
22. The probe mix of claim 19, wherein said one or more helper oligonucleotides include first and second helper oligonucleotides, wherein the target sequence of said first helper oligonucleotide is selected from the group consisting of SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25 and SEQ ID NO:27, and wherein the target sequence of said second helper oligonucleotide is selected from the group consisting of SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26 and SEQ ID NO:28.

20
23. An amplification primer for use in amplifying a nucleic acid sequence present in nucleic acid derived from a *Cryptosporidium* organism under amplification conditions, said primer comprising an oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in a target sequence selected from the group consisting of SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67 and SEQ ID NO:68,

wherein said primer optionally includes a 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

24. The primer of claim 23, wherein the target sequence is selected from
5 the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63.

25. The primer of claim 23, wherein the target sequence is selected from
the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64.

10 26. The primer of claim 23, wherein the target sequence is selected from
the group consisting of SEQ ID NO:47, SEQ ID NO:53, SEQ ID NO:59 and SEQ ID NO:65.

15 27. The primer of claim 23, wherein the target sequence is selected from
the group consisting of SEQ ID NO:48, SEQ ID NO:54, SEQ ID NO:60 and SEQ ID NO:66.

28. The primer of claim 23, wherein the target sequence is selected from
the group consisting of SEQ ID NO:49, SEQ ID NO:55, SEQ ID NO:61 and SEQ ID NO:67.

20 29. The primer of claim 23, wherein the target sequence is selected from
the group consisting of SEQ ID NO:50, SEQ ID NO:56, SEQ ID NO:62 and SEQ ID NO:68.

30. The primer of claim 23, wherein said oligonucleotide has an at least
10 contiguous base region which is at least 90% complementary to an at least 10 contiguous
base region present in the target sequence.

25 31. The primer of claim 23, wherein said oligonucleotide has an at least
10 contiguous base region which is 100% complementary to an at least 10 contiguous base
region present in the target sequence.

32. The primer of claim 23, wherein said primer is from 18 to 40 bases in length and optionally includes the 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

5 33. The primer of claim 23, wherein said primer includes the 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

10 34. The primer of claim 33, wherein the 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase is a T7 promoter having the base sequence of SEQ ID NO:69.

15 35. The primer of claim 23, wherein said primer contains base sequences which hybridize to each other when not hybridized to the target sequence under the amplification conditions.

36. The primer of claim 35 further comprising a group of interacting labels.

20 37. The primer of claim 36, wherein said interacting labels include a luminescent label and a quencher label. .

25 38. An amplification primer for use in amplifying a nucleic acid sequence present in nucleic acid derived from a *Cryptosporidium* organism under amplification conditions, said primer comprising an oligonucleotide, wherein the base sequence of said oligonucleotide is at least 80% complementary to the base sequence of a target sequence selected from the group consisting of SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64,

SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67 and SEQ ID NO:68, and wherein said primer optionally includes a 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

5 39. An amplification primer for use in amplifying a nucleic acid sequence present in nucleic acid derived from a *Cryptosporidium* organism under amplification conditions, wherein the base sequence of said primer is at least 80% complementary to the base sequence of a target sequence selected from the group consisting of SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67 and SEQ ID NO:68, and wherein said oligonucleotide optionally includes a 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

10
15
20
25
30 40. An amplification primer for use in amplifying a nucleic acid sequence present in nucleic acid derived from a *Cryptosporidium* organism under amplification conditions, wherein the base sequence of said primer is fully complementary to the base sequence of a target sequence selected from the group consisting of SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67 and SEQ ID NO:68, and wherein said oligonucleotide optionally includes a 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

35 41. A set of amplification primers for use in amplifying a nucleic acid sequence present in nucleic acid derived from a *Cryptosporidium* organism under

amplification conditions, wherein at least two primers of said set of primers comprise an oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in a target sequence selected from the group consisting of SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67 and SEQ ID NO:68, wherein one or more primers of said set of primers optionally include a 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30

42. The method of claim 41, wherein said set of primers includes:
a first primer, wherein the target sequence of said first primer is selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63; and

a second primer, wherein the target sequence of said second primer is selected from the group consisting of SEQ ID NO:47, SEQ ID NO:53, SEQ ID NO:59 and SEQ ID NO:65.

43. The method of claim 41, wherein said set of primers includes:
a first primer, wherein the target sequence of said first primer is selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63; and

a second primer, wherein the target sequence of said second primer is selected from the group consisting of SEQ ID NO:48, SEQ ID NO:54, SEQ ID NO:60 and SEQ ID NO:66.

44. The method of claim 41, wherein said set of primers includes:
a first primer, wherein the target sequence of said first primer is selected from the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64;

and

a second primer, wherein the target sequence of said second primer is selected from the group consisting of SEQ ID NO:47, SEQ ID NO:53, SEQ ID NO:59 and SEQ ID NO:65.

5

45. The method of claim 41, wherein said set of primers includes:

a first primer, wherein the target sequence of said first primer is selected from the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64; and

10

a second primer, wherein the target sequence of said second primer is selected from the group consisting of SEQ ID NO:48, SEQ ID NO:54, SEQ ID NO:60 and SEQ ID NO:66.

15

46. The method of claim 41, wherein said set of primers includes:

a first primer, wherein the target sequence of said first primer is selected from the group consisting of SEQ ID NO:49, SEQ ID NO:55, SEQ ID NO:61 and SEQ ID NO:67; and

20

a second primer, wherein the target sequence of said second primer is selected from the group consisting of SEQ ID NO:50, SEQ ID NO:56, SEQ ID NO:62 and SEQ ID NO:68.

25

47. An oligonucleotide for use in determining the presence of a *Cryptosporidium* organism in a test sample, said oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in a target sequence contained in nucleic acid derived from a *Cryptosporidium* organism, wherein the target sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63,

30

SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67 and SEQ ID NO:68, and wherein said oligonucleotide optionally includes a 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

5 48. An oligonucleotide for use in determining the presence of a *Cryptosporidium* organism in a test sample, wherein the base sequence of said oligonucleotide is at least 80% complementary to the base sequence of a target sequence present in nucleic acid derived from a *Cryptosporidium* organism, wherein the target sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67 and SEQ ID NO:68, and wherein said oligonucleotide optionally includes a 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

10 49. An oligonucleotide for use in determining the presence of a *Cryptosporidium* organism in a test sample, wherein the base sequence of said oligonucleotide is fully complementary to the base sequence of a target sequence present in nucleic acid derived from a *Cryptosporidium* organism, wherein the target sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67 and SEQ ID NO:68, and wherein said oligonucleotide optionally includes a 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

50. A method for determining the presence of a *Cryptosporidium* organism in a test sample, said method comprising the steps of:

contacting the test sample with said probe of claim 1 under stringent conditions; and

5 determining whether a probe:target hybrid has formed as an indication of the presence of a *Cryptosporidium* organism in the test sample.

51. A method for determining the presence of a *Cryptosporidium* organism in a test sample, said method comprising the steps of:

10 contacting the test sample with said probe of claim 16 under stringent conditions; and

determining whether a probe:target hybrid has formed as an indication of the presence of a *Cryptosporidium* organism in the test sample.

15 52. A method for determining the presence of a *Cryptosporidium* organism in a test sample, said method comprising the steps of:

contacting the test sample with said probe of claim 17 under stringent conditions; and

20 determining whether a probe:target hybrid has formed as an indication of the presence of a *Cryptosporidium* organism in the test sample.

53. A method for determining the presence of a *Cryptosporidium* organism in a test sample, said method comprising the steps of:

25 contacting the test sample with said probe of claim 18 under stringent conditions; and

determining whether a probe:target hybrid has formed as an indication of the presence of a *Cryptosporidium* organism in the test sample.

30 54. A method for amplifying *Cryptosporidium* nucleic acid which may be present in a test sample, said method comprising the steps of:

contacting the test sample with said primer of claim 23 under amplification conditions; and

amplifying a target sequence present in nucleic acid derived from a *Cryptosporidium* organism which may be present in the test sample.

5

55. The method of claim 54, wherein the target sequence is selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63.

10 56. The method of claim 54, wherein the target sequence is selected from the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64.

57. The method of claim 54, wherein the target sequence is selected from the group consisting of SEQ ID NO:47, SEQ ID NO:53, SEQ ID NO:59 and SEQ ID NO:65.

15 58. The method of claim 54, wherein the target sequence is selected from the group consisting of SEQ ID NO:48, SEQ ID NO:54, SEQ ID NO:60 and SEQ ID NO:66.

59. The method of claim 54, wherein the target sequence is selected from the group consisting of SEQ ID NO:49, SEQ ID NO:55, SEQ ID NO:61 and SEQ ID NO:67.

20

60. The method of claim 54, wherein the target sequence is selected from the group consisting of SEQ ID NO:50, SEQ ID NO:56, SEQ ID NO:62 and SEQ ID NO:68.

25

61. The method of claim 54 further comprising the step of determining the presence of the amplified target sequence in the test sample with a hybridization assay probe.

30

62. The method of claim 61, wherein said probe comprises an oligonucleotide which hybridizes to the amplified target sequence under stringent conditions to form a probe:target hybrid stable for detection, said oligonucleotide having an at least 10

contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in the amplified target sequence, wherein the amplified target sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, and wherein said probe does not hybridize to nucleic acid derived from a non-
5 *Cryptosporidium* organism in the test sample to form a probe:non-target hybrid stable for detection under the stringent conditions.

63. A method for amplifying *Cryptosporidium* nucleic acid which may be present in a test sample, said method comprising the steps of:

10 contacting the test sample with said set of primers of claim 41 under amplification conditions; and

amplifying a target sequence present in nucleic acid derived from a
15 *Cryptosporidium* organism which may be present in the test sample.

64. The method of claim 63, wherein said set of primers includes:

a first primer, wherein the target sequence of said first primer is selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63;
20 and

a second primer, wherein the target sequence of said second primer is selected from the group consisting of SEQ ID NO:47, SEQ ID NO:53, SEQ ID NO:59 and SEQ ID NO:65.

65. The method of claim 63, wherein said set of primers includes:

a first primer, wherein the target sequence of said first primer is selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63;
25 and

a second primer, wherein the target sequence of said second primer is selected from the group consisting of SEQ ID NO:48, SEQ ID NO:54, SEQ ID NO:60 and SEQ ID NO:66.

66. The method of claim 63, wherein said set of primers includes:
a first primer, wherein the target sequence of said first primer is selected from
the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64;
and

5 a second primer, wherein the target sequence of said second primer is
selected from the group consisting of SEQ ID NO:47, SEQ ID NO:53, SEQ ID NO:59 and
SEQ ID NO:65.

10 67. The method of claim 63, wherein said set of primers includes:
a first primer, wherein the target sequence of said first primer is selected from
the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64;
and

15 a second primer, wherein the target sequence of said second primer is
selected from the group consisting of SEQ ID NO:48, SEQ ID NO:54, SEQ ID NO:60 and
SEQ ID NO:66.

20 68. The method of claim 63, wherein said set of primers includes:
a first primer, wherein the target sequence of said first primer is selected from
the group consisting of SEQ ID NO:49, SEQ ID NO:55, SEQ ID NO:61 and SEQ ID NO:67;
and

25 a second primer, wherein the target sequence of said second primer is
selected from the group consisting of SEQ ID NO:50, SEQ ID NO:56, SEQ ID NO:62 and
SEQ ID NO:68.

69. The method of claim 63 further comprising the step of determining
the presence of the amplified target sequence in the test sample with a hybridization assay
probe.

30 70. The method of claim 69, wherein said probe comprises an
oligonucleotide which hybridizes to the amplified target sequence under stringent conditions

to form a probe:target hybrid stable for detection, said oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in the amplified target sequence, wherein the amplified target sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4, and wherein said probe does not hybridize to nucleic acid derived from a non-*Cryptosporidium* organism in the test sample to form a probe:non-target hybrid stable for detection under the stringent conditions.

71. A method for amplifying *Cryptosporidium* nucleic acid which may be present in a test sample, said method comprising the steps of:

contacting the test sample with said primer of claim 38 under amplification conditions; and

amplifying a target sequence present in nucleic acid derived from a *Cryptosporidium* organism which may be present in the test sample.

72. A method for amplifying *Cryptosporidium* nucleic acid which may be present in a test sample, said method comprising the steps of:

contacting the test sample with said primer of claim 39 under amplification conditions; and

amplifying a target sequence present in nucleic acid derived from a *Cryptosporidium* organism which may be present in the test sample.

73. A method for amplifying *Cryptosporidium* nucleic acid which may be present in a test sample, said method comprising the steps of:

contacting the test sample with said primer of claim 40 under amplification conditions; and

amplifying a target sequence present in nucleic acid derived from a *Cryptosporidium* organism which may be present in the test sample.

74. A kit comprising, in packaged combination, two or more oligonucleotides for use in determining the presence of a *Cryptosporidium* organism in a test sample, each of said oligonucleotides having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in a target sequence contained in nucleic acid derived from a *Cryptosporidium* organism, wherein the target sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67 and SEQ ID NO:68, and wherein one or more of said oligonucleotides optionally includes a 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

75. The kit of claim 74, wherein said two or more oligonucleotides include:

a first oligonucleotide, wherein the target sequence of said first oligonucleotide is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4; and

a second oligonucleotide, wherein the target sequence of said second oligonucleotide is selected from the group consisting of SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67 and SEQ ID NO:68.

76. The kit of claim 75, wherein the target sequence of said second oligonucleotide is selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63.

77. The kit of claim 75, wherein the target sequence of said second oligonucleotide is selected from the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64.

5 78. The kit of claim 75, wherein the target sequence of said second oligonucleotide is selected from the group consisting of SEQ ID NO:47, SEQ ID NO:53, SEQ ID NO:59 and SEQ ID NO:65.

10 79. The kit of claim 75, wherein the target sequence of said second oligonucleotide is selected from the group consisting of SEQ ID NO:48, SEQ ID NO:54, SEQ ID NO:60 and SEQ ID NO:66.

15 80. The kit of claim 75, wherein the target sequence of said second oligonucleotide is selected from the group consisting of SEQ ID NO:49, SEQ ID NO:55, SEQ ID NO:61 and SEQ ID NO:67.

81. The kit of claim 75, wherein the target sequence of said second oligonucleotide is selected from the group consisting of SEQ ID NO:50, SEQ ID NO:56, SEQ ID NO:62 and SEQ ID NO:68.

20 82. The kit of claim 74, wherein said two or more oligonucleotides include:

25 a first oligonucleotide, wherein the target sequence of said first oligonucleotide is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4;

a second oligonucleotide, wherein the target sequence of said second oligonucleotide is selected from the group consisting of SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:64 and SEQ ID NO:67; and

a third oligonucleotide, wherein the target sequence of said third oligonucleotide is selected from the group consisting of SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:65, SEQ ID NO:66 and SEQ ID NO:68.

5

83. The method of claim 82, wherein:
the target sequence of said second oligonucleotide is selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63; and
wherein the target sequence of said third oligonucleotide is selected from the group consisting of SEQ ID NO:47, SEQ ID NO:53, SEQ ID NO:59 and SEQ ID NO:65.

10

84. The method of claim 82, wherein:
the target sequence of said second oligonucleotide is selected from the group consisting of SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:57 and SEQ ID NO:63; and
the target sequence of said third oligonucleotide oligonucleotide is selected from the group consisting of SEQ ID NO:48, SEQ ID NO:54, SEQ ID NO:60 and SEQ ID NO:66.

15

85. The method of claim 82, wherein:
the target sequence of said second oligonucleotide is selected from the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64; and
the target sequence of third oligonucleotide is selected from the group consisting of SEQ ID NO:47, SEQ ID NO:53, SEQ ID NO:59 and SEQ ID NO:65.

20

86. The method of claim 82, wherein:
the target sequence of said second oligonucleotide is selected from the group consisting of SEQ ID NO:46, SEQ ID NO:52, SEQ ID NO:58 and SEQ ID NO:64.; and
the target sequence of said third oligonucleotide is selected from the group consisting of SEQ ID NO:48, SEQ ID NO:54, SEQ ID NO:60 and SEQ ID NO:66.

25

30

87. The method of claim 82, wherein:

the target sequence of said second oligonucleotide is selected from the group consisting of SEQ ID NO:49, SEQ ID NO:55, SEQ ID NO:61 and SEQ ID NO:67; and

the target sequence of said third oligonucleotide is selected from the group consisting of SEQ ID NO:50, SEQ ID NO:56, SEQ ID NO:62 and SEQ ID NO:68.

5

88. A kit comprising, in packaged combination, said probe of claim 1 and at least one helper oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in a target sequence selected from the group consisting of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27 and SEQ ID NO:28.

10

89. A kit comprising, in packaged combination, two or more amplification primers, each said primer comprising an oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in a target sequence selected from the group consisting of SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67 and SEQ ID NO:68, wherein each said primer optionally includes a 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

20

90. A kit comprising, in packaged combination, said probe of claim 1 and an amplification primer comprising an oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in a target sequence selected from the group consisting of SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID

25

30

NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67 and SEQ ID NO:68, wherein said primer optionally includes a 5' sequence which is recognized by an RNA polymerase or which enhances initiation or elongation by an RNA polymerase.

5 91. The kit of claim 90 further comprising at least one helper oligonucleotide having an at least 10 contiguous base region which is at least 80% complementary to an at least 10 contiguous base region present in a target sequence selected from the group consisting of SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27 and SEQ ID NO:28.

10

PROCESSED 8/23/07